

## Comparative Cytotoxic Effects of the Hydrosols of some Ethnobotanic Plants

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<https://doi.org/10.38093/cupmap.765089>

Received : 06/07/2020

Accepted : 08/12/2020

**Abstract**

Hydrosols are aqueous solutions and by-products of the essential oil distillation procedure. They have many medicinal benefits because contain the water-soluble components, vitamins and minerals of the plant that has been distilled. On the other hand, colorectal cancer (CRC) is the third most common cancer in both men and women worldwide and is the main cause of death in gastrointestinal cancer. Hence in this study, the cytotoxic effects of hydrosols obtained from five different ethnobotanical plants (*Melissa officinalis* L., *Achillea teretifolia* Willd., *Achillea aleppica* subsp. *zederbaueri* (Hayek) Hub.-Mor., *Origanum onites* and *Salvia fruticosa* Mill) have been investigated on the colorectal cancer cell line. For the determination of the hydrosols cytotoxic effect MTT assay was used. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is used to determine the cytotoxic or cytostatic effects of plant extracts, potential medicinal agents and toxic materials. Based on the results of the cytotoxicity assay, the most effective extract was obtained from *Origanum onites* and the next effective extract from *Melissa officinalis*. On the other hand, both *Achillea* extracts showed low cytotoxic activity. Consequently, this study provides some criticism on the potentials of some plants traditionally used as an anticancer agent, and also shows that there is more need to investigate the cause of cell death and active substances.

**Key Words:** Cytotoxicity, Hydrosols, Lemon balm, Turkish oregano, CRC.

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**1. Introduction**

Ethnobotanical researches contribute significantly to the scientific evaluation of plants with their content, which has been acquired through trial and error and reflects valuable information that has been passed down from generation to generation as a result of a long period of time (Kendir & Güvenç 2010). Our country, which has a rich cultural heritage, also has an extensive ethnobotanical information treasure. The use of plants as complementary medicine for

cancer, especially in difficult cancer types, has recently increased.

Cancer is an important public health problem and causes serious pain and economic losses worldwide. Colorectal cancer is the third most common cancer in the world. Therefore, more research is needed to develop safe products for the prevention and treatment of all human cancers. Approximately 60% of the drugs currently used for cancer treatment have been isolated from natural products (Gordaliza, 2009), and the plant kingdom

has been the primary source. Wild sources of medicinal plants have been used by humans for centuries in traditional healing systems. Indigenous people have adopted different forms of application and use to take advantage of this natural resource (Adnan et al., 2014).

*Melissa officinalis* L. (Lamiaceae) is one of the most used medicinal plants in Europe and the Mediterranean region, as a herbal tea for their aromatic, digestive and antispasmodic properties in nervous disturbance of sleep and functional gastrointestinal disorders (Bisset, 1994). *Achillea* L. (civanperçemi; Turkish name) is a medicinal plant genus which has been used since ancient times. It possesses diversity all around the world. The majority of the *Achillea* species are important native economic plants of Anatolia. In Turkey, herbal teas prepared from some *Achillea* species are used in folk medicine for abdominal pain, diarrhea, and flatulence as well as a diuretic and emmenagog. Some investigations about anti-oxidant and cytotoxic effects of *Achillea* species have been performed. The extracts or essential oils of *Achillea wilhelmsii* C. Koch, *A. micrantha* Willd., *A. millefolium* L., *A. pannonica* Scheele and *A. fragrantissima* (Forssk.) Sch. Bip. has been reported to be natural antioxidant sources (Souri et al., 2004; Nickavar et al., 2006; Wojdylo et al., 2007; Bozin et al., 2008; Tarawneh et al., 2010). Also, *Achillea millefolium* L., *A. clavennae* L., *A. talagonica* Bioss., *A. wilhelmsii* C. Koch and *A. fragrantissima* are important species with cytotoxic effects (Sathiyamoorthy et al., 1999; Trifunovic et al., 2006; Saeidnia et al., 2009; Ali et al., 2011; Li et al. 2011; Bali et al. 2015; Amini Navaie et al., 2015; Köngül et al., 2017). *Origanum onites* L., known as Turkish oregano, is a traditional and medicinal herb. Infusions obtained from aerial parts are effective in the treatment of several gastrointestinal diseases, influenza, bronchitis, hypertension, diabetes, high

cholesterol, stomach disorders and leukaemia (Sivas and Tomsuk 2011; Sargin et al., 2013; Gürdal & Kültür 2013). *Salvia fruticosa*, insufficiently studied for various activities, is a Mediterranean medicinal and aromatic herb. It is known for its antioxidant, antimicrobial and antiproliferative activities (Alimpic et al., 2015; Altay & Bozoğlu 2017). Essential oils are usually extracted from plants by steam distillation, where an aqueous phase called hydrosol is obtained. Unlike essential oils, hydrosol studies are limited, despite the interest of the food, cosmetic and phytotherapeutic industries to find a natural protective alternative (Tornuk et al., 2011; Hamedi et al., 2017). Hydrosol is highly valuable as it contains all the water-soluble substances of the plant.

The present study was designed to investigate cytotoxic effects of the hydrosols of five ethno-botanic plants (*Melissa officinalis* L., *Achillea teretifolia* Willd., *Achillea aleppica* subsp. *zederbaueri* (Hayek) Hub.-Mor., *Origanum onites* and *Salvia fruticosa* Mill.) on the colorectal cell line.

## 2. Material and Method

### 2.1. Plant material

In our study, investigated plant samples were provided from Eray TULUKÇU. Plant samples were grown at Selcuk University, Çumra Vocational School, Medical and Aromatic Plants Department. Hydrosol preparations were performed the protocol of Linskens (1997). The hydrosols were kept in refrigerated at 4°C until they are used. The extracts coded as *Melissa officinalis* (MOE) *Achillea teretifolia* (ATE), *Achillea aleppica* subsp. *zederbaueri* (AAE), *Origanum onites* (ORE) and *Salvia fruticosa* (SFE), respectively.

### 2.2. Cell lines

Human colon adenocarcinoma cells, DLD1 was obtained from ATCC and they were cultured in 10% (v/v) heat-inactivated fetal

bovine serum (FBS), 1 % (v/v) penicillin-streptomycin supplemented RPMI 1640 medium. Cells were incubated at 37° C under 5% CO<sub>2</sub> conditions. After reaching 70% confluency, the cells were trypsinized, counted and transferred in 96-well plates.

### 2.3. Cytotoxicity assays

The cytotoxic activity of the hydrosols was tested on the DLD1 cell line. Hydrosols were prepared in six different dilutions (v/v) and were applied to the cells. Different amounts of the hydrosol were prepared for cell treatment by diluting the stock with medium only. Then the plates were incubated for 24 - 48 hours. The cell proliferation assay was carried out via MTT (5mg/ml). The optical density of the plates was measured using the Elisa microplate reader at 540 nm. Each experiment was performed three times and the mean values were taken into consideration.

### 2.4. Statistical Analysis

For statistical analysis of the data, multiple comparisons were made using one-way variance analysis (ANOVA) followed by Dunnett's test for post hoc analysis. The differences in  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  were considered statistically significant.

## 3. Results and Discussion

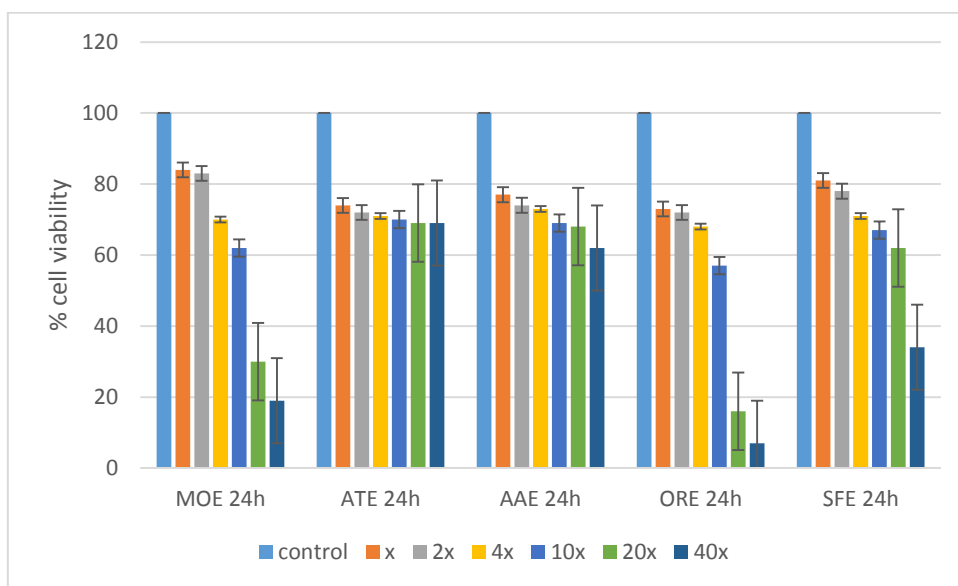
As a result of the link between man and plant that has been going on for centuries, the ethnobotanical discipline, which is considered important all over the world, was born. Ethnobotanical research contributes to the scientific evaluation of plants by providing valuable information obtained through experimentation and passed down from generation to generation. The use of medicinal plants for thousands of years has played a major role in the emergence of ethnobotany for the treatment of various diseases such as cancer. By the World Health Organization (WHO) data (WHO, 2001), 80%

of the world population prefer herbal medicines in the treatment of various diseases (Chikezie & Ojiako, 2015; Msomi and Simelane, 2018; Nguyen et al., 2020; Khan et al., 2020). With this situation, ethnobotanical knowledge transferred from the early ages comes to the fore and the use of medicinal plants becomes important (Bozyel & Merdamert, 2018). Turkey, which has hosted many civilizations, constitutes a rich cultural heritage and rich research environment in terms of ethnobotanical studies. Medicinal plants are vital sources of readily accessible remedy used in the countryside healthcare system. Since cancer is a leading cause of death, various researches are carried out in this field every day worldwide. These studies usually involve investigating the effects of biologically active substances on cancer cells and are often derived from plants (Mukherjee et al. 2001). There is a great need to examine the sources of safe and inexhaustible natural substances. It is also important to understand the mechanisms of anticancer agents for future application in cancer treatment (Half et al. 2009). Increasing evidence suggests that extracts and/or active ingredients from plants are effective against cancer by preventing carcinogenesis and tumour progression or by killing cancer cells (Demir et al., 2020).

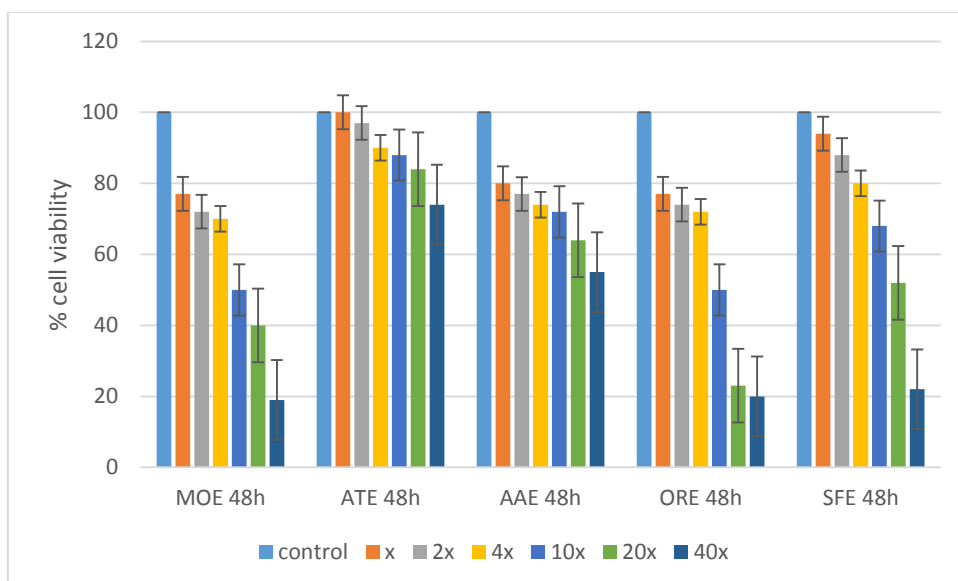
Although the cytotoxic effects of the plants preferred in our study have been previously examined in the several cell lines such as colon, breast, prostate and liver cell lines. As far as we know our study is a first in terms of the cell line used in our study. This is the first study to reveal the cytotoxic effect of hydrosols from various ethnobotanical plants on colorectal cancer cell line DLD1. According to the cytotoxicity test for cells treated by *Melissa officinalis*, *Achillea teretifolia*, *Achillea aleppica* subsp *zederbaueri*, *Origanum onites* and *Salvia fruticosa* aqueous extracts, our results show that the cytotoxicity for the DLD1 cell line

between the applied dose ranges. From the results of MTT, the most effective extract is *Origanum onites*. On the other hand, two *Achillea* extracts exhibited less cytotoxic effects than others in the doses and time intervals administered. The concentration of 50% cellular cytotoxicity of extracts on cancer cells (IC<sub>50</sub>) was based on 48-hour absorbance values (Chen et al., 2009). The

IC<sub>50</sub> dose is approximately 25% for *Melissa officinalis* (MOE), 25% for *Origanum onites* (ORE) and 50% for *Salvia fruticosa* (SFE). The cytotoxicity graphs of 24-48h treatments were given Figure 1 and Figure 2. Different solvents and extraction methods can be tried to determine the cytotoxic effects of *Achillea* extracts.



**Fig.1.** Graph of MTT assay after 24h treatment (40x: direct, 20x: 1/2 (v/v), 10x: 1/4 (v/v), 4x: 1/10 (v/v) 2x:1/20(v/v) x: 1/40 (v/v))



**Fig.2.** Graph of MTT assay after 48h treatment (40x: 1, 20x: 1/2 (v/v), 10x: 1/4 (v/v), 4x: 1/10 (v/v) 2x:1/20(v/v) x: 1/40 (v/v))

Colorectal cancer is the second most frequently diagnosed in women, the third in men and fourth cancer worldwide (Jemal et al., 2011). Colorectal cancer, which is reported to cause death in approximately 60% of cases, is common in developed countries (Ferlay et al., 2015; Merika et al., 2015). Herbal extracts or preparations inhibit colorectal cancer cells by stimulating apoptosis or autophagy, inducing the cell cycle and triggering signal pathways. There are many studies on the cytotoxic and apoptotic effects of plants used in our study on various colon cancer cell lines, but as far as we know, no previous studies have been performed on the DLD1 cell line. The ethanolic and aqueous extracts from *Melissa officinalis* have been reported to have cytotoxic effects on human colon cancer cell line HCT-116 (Encalada et al., 2011). The hydroalcoholic extract from *Melissa officinalis* has been reported to inhibit apoptosis and cell proliferation in HT29 and T84 colon cancer cells (Weidner et al., 2015). In another study, it was reported that essential oil of *Origanum* and main component planned carvacrol has cytotoxic and apoptotic activity on Hep-G2 cells and can be evaluated as potential anticancer agents (Sivas and Tomsuk 2011). Xavier et al. (2009) have been reported that *Salvia fruticosa* and *Salvia officinalis* water extracts and their main phenolic compound rosmarinic acid has antiproliferative and pro-apoptotic effects on two human colon cancer cell lines, HCT15 and C0115.

#### 4. Conclusion

In conclusion, this study gives some criticism on the potentials of some plants traditionally used in the field of pharmacology as an anticancer agent. This is a preliminary study and, in the future, it is aimed to find out the molecular mechanisms that cause cell death. Additionally, it is planned to prepare various extracts by using new solvents and different extraction techniques with the plants used in

the study and investigate their cytotoxic potential.

#### Acknowledgments

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#### Conflict of Interest

No conflict of interest was reported by the authors.

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